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ORIGINAL ARTICLE

Causality between smoking and female reproductive disorders: A Mendelian randomization study

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ABSTRACT

Background: Smoking may impair female reproductive health, but its causal role remains unclear. This study evaluated the causal associations of smoking initiation, lifetime smoking index, and smoking cessation with 15 female reproductive disorders. **Methods:** Linkage disequilibrium score regression (LDSC), Mendelian randomization (MR), and colocalization analysis assessed genetic correlations and causal associations, and detected shared genetic variants between smoking-related phenotypes and female reproductive disorders. **Results:** Genetic liability to smoking initiation was associated with higher risks of adenomyosis (odds ratio [OR] = 1.57, P = 0.008), dysplastic lesions involving the cervix, vagina, or vulva (OR = 1.34, P = 0.016), inflammatory disease of the uterus (OR = 1.52, P = 0.016), oligomenorrhea (OR = 2.09, P = 0.032), and torsion of ovary, ovarian pedicle, and fallopian tube (OR = 2.43, P = 0.046). A higher lifetime smoking index was associated with increased risks of dysplastic lesions involving the cervix, vagina, or vulva (OR = 1.55, P = 0.020), cervical dysplastic lesions (OR = 1.70, P = 0.007), excessive, frequent, and irregular menstruation (OR = 1.37, P = 0.003), and inflammatory disease of the uteri (OR = 2.66, P < 0.001). Genetic propensity to smoking cessation resulted in a reduced risk of endometriosis (OR = 0.58, P = 0.015). Colocalization revealed two shared genetic loci (inhibitory synaptic factor 1 [*INSYN1*] and phosphodiesterase 4B [*PDE4B*]) between lifetime smoking index and inflammatory disease of the uterus. **Conclusion:** These findings provide genetic evidence that smoking-related behaviors may causally influence several female reproductive disorders.

Keywords: smoking, female reproductive disorders, Mendelian randomization, colocalization, single-nucleotide polymorphisms

INTRODUCTION

In recent years, the increasing prevalence of smoking among females has been recognized as a global health challenge.[1] Cigarette smoke contains over 7000 toxic chemicals, including nicotine, polycyclic aromatic hydrocarbons, and heavy metals, which have been shown to cause reproductive toxicity,[2,3] and affect ovarian reserve, steroidogenesis, ovulation, tubal functions, and endometrial receptivity.[4–7] Observational studies have identified smoking as a risk factor for several female reproductive system disorders, including inflammatory

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disease of the uteri, polycystic ovarian syndrome (PCOS), irregular menstruation, and early menopause.[8–10] However, a causal relationship between smoking and female reproductive disorders is yet to be ascertained, as confounding factors and reverse causal relationships are limitations of observational studies.[11]

The Mendelian randomization (MR) method is widely used in the field of epidemiology. It circumvents the influence of confounding factors and reverse causality and enables exploration of the causal relationship between exposure factors and outcome variables.[12] Moreover, MR does not require absolute control and experimental groups as in the randomized control method and can avoid potential ethical issues.[13]

In this study, a two-sample MR was applied to analyze the effect of smoking on 15 female reproductive disorders according to three phenotypes: initiation, lifetime smoking and cessation, as further evidence of a causal relationship between smoking and female reproductive disorders.

MATERIALS AND METHODS

Study design

This was a two-sample MR study aimed at identifying the impact of smoking on female reproductive disorders (Figure 1). Given that our data were at a summary level and did not involve any personal or private information, ethical approval was deemed unnecessary.

Data source for exposure

Smoking behavior was assessed across three dimensions: smoking initiation, smoking cessation, and smoking severity. Smoking initiation was examined as a binary variable indicating whether an individual had ever been a regular smoker. Smoking cessation was evaluated through a binary variable comparing current and former smokers. Both indicators were derived from a meta-genome-wide association study (GWAS) analysis provided by the GWAS and sequencing consortium of alcohol and nicotine use[14] (https://genome.psych.umn.edu/index.php/; GWAS and Sequencing Consortium of [GSCAN]) with over 1,232,091 and 547,219 individuals, respectively. For a more accurate assessment of smoking severity, we employed a lifetime smoking index derived from GWAS data from the UK Biobank[15] (http://www.ukbiobank.ac.uk), which included 462,690 individuals. Approximately 30% of participants had a history of smoking (8% were current smokers and 22% were former smokers). The standard deviation of the lifetime smoking index was equivalent to smoking 20 cigarettes per day for 15 years, followed by quitting 17 years ago, or smoking 60 cigarettes per day for 13 years, and quitting 22 years ago.

Genetic instrument selection and MR assumptions

The MR analysis relies on the fulfillment of three critical assumptions.[16] Firstly, the instrument should demonstrate a robust correlation with the exposure. Secondly, the genetic variants selected as instruments should not be associated with any other phenotypes that may introduce bias into the association between the exposure and the outcome. Lastly, the genetic variants should not be linked with the outcome *via* any other pathways aside from the exposure. To ensure that these assumptions are met, we selected strongly correlated and independent single-nucleotide polymorphisms (SNPs) as instrumental variables (IVs) ($P < 5 \times 10^{-8}$, $r^2 = 0.001$, clumping window size of 10 MB), with all SNPs having a minor allele frequency > 0.01 and F-statistic > 10. Additionally, we used Phenoscanner to eliminate SNPs that may have horizontal pleiotropy and those highly linked with the outcome were removed ($P < 0.05$). To summarize, we identified 200,126, and 19 effective SNPs for smoking initiation, smoking cessation, and lifetime smoking index, respectively.

Data source for outcome

The GWAS data related to female reproductive disorders were sourced from the R7 edition of the FinnGen, released in June 2022. The cohort consists of over 170,000 women and underwent a comprehensive analysis of 16,962,023 genetic variations. This study includes 15 female reproductive disorders.

Linkage disequilibrium score regression (LDSC)

LDSC is a robust tool for genetically correlating complex traits or diseases.[17] It can help distinguish between true polygenes and mixed biases, such as implicit association and demographic stratification. It is a particularly effective tool for large sample sizes.[18] In this study, we utilized LDSC[19] to estimate the heritability by univariate LDSC and the genetic correlation between smoking and female reproductive disorders by bivariate LDSC. The summary statistics were filtered based on HapMap3 and SNPs that were ambiguous or had a minor allele frequency < 0.01 were excluded; pre-computed linkage disequilibrium (LD) scores and weights for the European population, derived from 1000 Genomes, were used.

MR analysis

The random-effects inverse-variance weighted (IVW) method was used as our primary MR method to assess the causal effects of the three dimensions of smoking on female reproductive disorders. However, it is imperative to acknowledge that the outcomes of the IVW method may be compromised by the horizontal pleiotropy of IVs. To improve the credibility of our findings, we

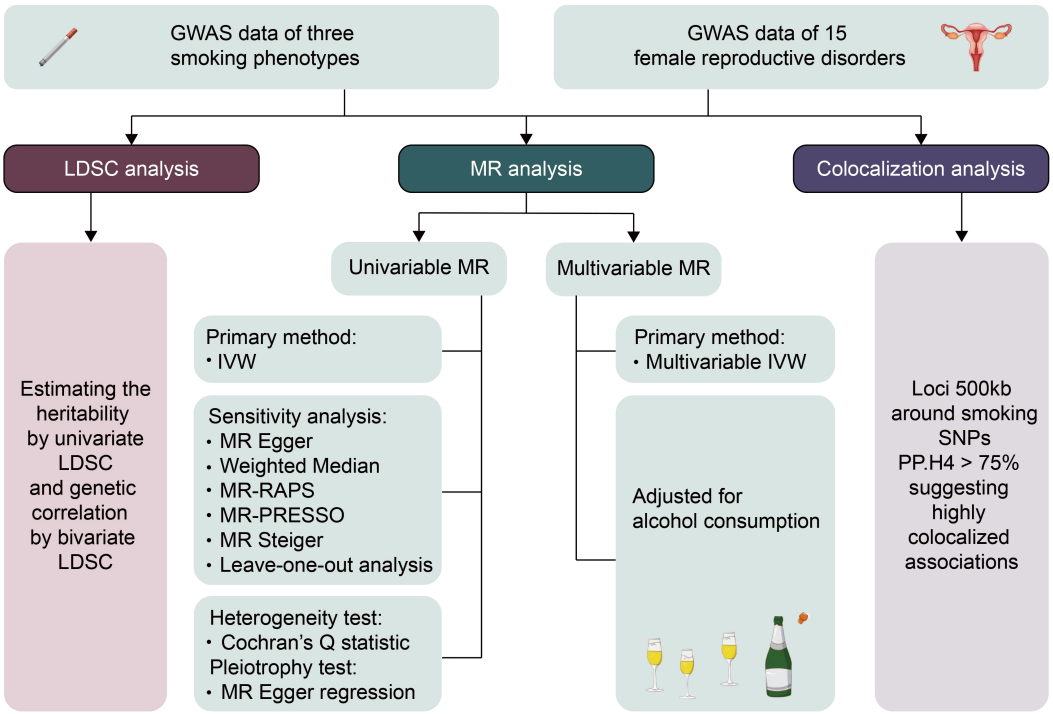

Figure 1. Study design flowchart. GWAS, genome-wide association study; LDSC, linkage disequilibrium score regression; MR, Mendelian randomization; IVW, inverse-variance weighted; MR-RAPS, Mendelian randomization-robust adjusted profile scoring; MR-PRESSO, Mendelian randomization-pleiotropy residual sum and outlier; PP.H4, posterior probability for hypothesis 4.

conducted multiple MR approaches, which included the weighted median, MR Egger regression, and MR-robust adjusted profile scoring (MR-RAPS). Of note, the weighted median method can accurately estimate causal relationships, even when 50% of IVs are invalid.[20] MR Egger regression is based on the instrument strength independent of the direct effect assumption to evaluate pleiotropy through the intercept term and a zero intercept implies no horizontal pleiotropy.[21,22] MR-RAPS aims to mitigate potential biases that could arise from violating certain assumptions inherent in MR analysis, such as the presence of horizontal pleiotropy and weak instruments.[23] To address the issue of reverse causality, where the smoke status is treated as the outcome and the female reproductive disorders are treated as the exposure, the Steiger filtering method was employed to determine the correct direction of inference.[24] Finally, Cochran's IVW Q statistic, and "leave-one-out" sensitivity analysis were performed to assess the heterogeneity and stability of the genetic variants. Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) was used to identify and eliminate any outlier variants, and to minimize heterogeneity and the potential influence of horizontal pleiotropy.

To account for the potential impact of multiple exposures that are causally related to the outcome, multivariable Mendelian randomization (MVMR) was performed using the "GagnonMR" package to accurately adjust for these exposures and minimize the risk of any biases.[25] The MVMR methods encompassed multivariable IVW, multivariable Median, multivariable least absolute shrinkage and selection operator (LASSO), and multivariable Egger.

The F-statistic[26–28] was utilized to assess the strength of the IVs in this study. It was calculated using the formula $F = \beta^2_{exposure}/SE^2_{exposure}$. An F-statistic > 10 indicates that weak instrumental bias is insignificant.

The ORs and corresponding 95% confidence intervals were calculated to assess the causal effects. P values < 0.05 were deemed significant. All analyses were two-sided and performed using R packages "TwoSampleMR" and "forestplot" in R 4.2.1.

Colocalization analysis

Colocalization analysis involves a series of mathematical calculations and statistical tests to explore the shared, local genetic architecture between two traits, and determine whether their observed overlap or spatial proximity is the result of mere chance.[29,30] This highly sophisticated analytical tool not only strengthens the associations identified in MR analysis but also helps identify potential confounding factors arising from LD, where a genetic variant in high LD with an IV may also be linked to the outcome of interest.[31] To carry out

colocalization analysis, complete GWAS data are imported, and the window size is set to ±500 kb, with the IVs as the center point. The default priors, with p1 as 1×10^{-4}, p2 as 1×10^{-4}, and p12 as 1×10^{-5}, are employed. The evidence for colocalization is evaluated using the posterior probability for hypothesis 4 (PP.H4), which indicates that both smoking and female reproductive disorders are associated with and driven by the same causal variant. A threshold of PP.H4 > 75% is utilized to suggest highly colocalized associations. All analyses were performed using R packages "coloc".

RESULTS

A rigorous selection process was conducted to identify 200,126, and 19 SNPs with *F*-statistics exceeding 10, which were independent and strongly correlated ($P < 5 \times 10^{-8}$, $r^2 = 0.001$, clumping window size of 10 MB) with smoking initiation, lifetime smoking index, and smoking cessation at the genome-wide level, respectively (Supplementary Table 1-3). These SNPs were subsequently employed in MR to investigate the causal relationship between the three smoking phenotypes and the 15 female reproductive disorders.

Genetic correlation and causal effect between smoking initiation and female reproductive disorders

Our initial exploration sought to unearth the plausible relationship between smoking initiation and female reproductive disorders. Through meticulous LDSC analysis, we uncovered a consequential positive correlation between smoking initiation and the onset of five various afflictions (Figure 2; Supplementary Table 4), including all dysplastic lesions of the cervix uteri, vagina, or vulva ($r_g = 0.31$, $P < 0.001$), all dysplastic lesions of the cervix uteri ($r_g = 0.30$, $P < 0.001$), adenomyosis ($r_g = 0.18$, $P < 0.001$), excessive, frequent and irregular menstruation ($r_g = 0.17$, $P < 0.001$), and inflammatory disease of the uteri ($r_g = 0.45$, $P < 0.001$). We then performed MR analysis to corroborate and affirm these preliminary findings.

The MR analysis results unequivocally demonstrated an increased risk of five female reproductive disorders with smoking initiation (Figure 3), including adenomyosis (endometriosis of uteri; OR = 1.57, $P = 0.008$), all dysplastic lesions of the cervix uteri, vagina, or vulva (OR = 1.34, $P = 0.016$), inflammatory disease of the uteri (OR = 1.52, $P = 0.016$), oligomenorrhea (OR = 2.09, $P = 0.032$), and torsion of ovary, ovarian pedicle, and fallopian tube (OR = 2.43, $P = 0.046$). Notably, all MR-RAPS findings were consistently significant ($P < 0.05$ for all; Supplementary Table 5), while the weighted median analysis also revealed positive results for oligomenorrhea ($P = 0.034$). In addition, the four distinct MR analysis approaches, including IVW, MR Egger, weighted median, and MR-RAPS, all demonstrated consistent directions, further bolstering the validity of our findings.

Genetic correlation and causal effect between lifetime smoking index and female reproductive disorders

The bivariate LDSC analysis indicated a positive genetic correlation between lifetime smoking index and nine female reproductive disorders (Figure 3). Among them, the genetic correlation between lifetime smoking index and inflammatory disease of the uteri was the most notable, with a genetic correlation coefficient of 0.59 ($P < 0.001$; Supplementary Table 6).

MR results showed that the lifetime smoking index may increase the risk of four female reproductive disorders (Figure 4), including all dysplastic lesions of the cervix uteri, vagina, or vulva (OR = 1.55; $P = 0.020$), all dysplastic lesions of the cervix uteri (OR = 1.70, $P = 0.007$), excessive, frequent, and irregular menstruation (OR = 1.37, $P = 0.003$), and inflammatory disease of the uteri (OR = 2.66, $P < 0.001$; Figure 3). Compared to smoking initiation, the OR values for the relationships between lifetime smoking index and all dysplastic lesions of the cervix uteri, vagina, or vulva and inflammatory disease of the uteri were greater, indicating a more profound influence.

Moreover, the weighted median and MR-RAPS results for these diseases were consistent with IVW. MR Egger revealed a significant causal relationship between the lifetime smoking index and all dysplastic lesions of the cervix uterine, vagina, or vulva (OR = 6.17, $P = 0.020$; Supplementary Table 7). However, the results of MR Egger were not consistent with the other three methods regarding the causal link between lifetime smoking index and excessive, frequent, and irregular menstruation. Therefore, this correlation was of suggestive significance.

Causal effect between smoking cessation and female reproductive disorders

Given the previous research and our findings, we found a significant positive genetic correlation between smoking cessation and smoking initiation, possibly because the GWAS sample for smoking cessation was composed of individuals who had ever smoked. Therefore, LDSC analysis of smoking cessation may be confounded. Moreover, these two phenotypes of smoking did not share any SNP. Consequently, we only conducted MR analysis to investigate the impact of smoking cessation on female reproductive disorders.

A negative correlation was identified between smoking

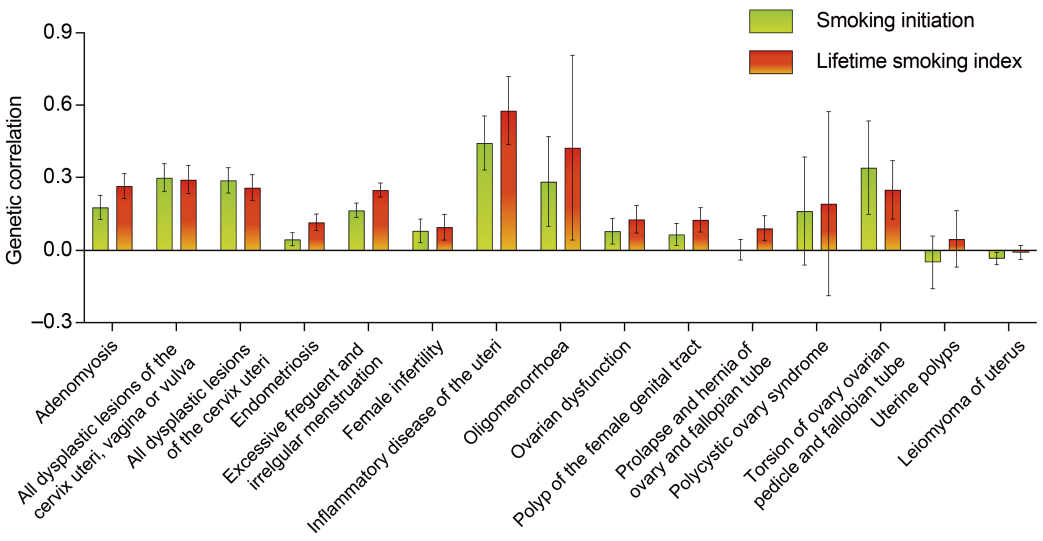

Figure 2. Genetic correlation for smoke and female reproductive disorders.

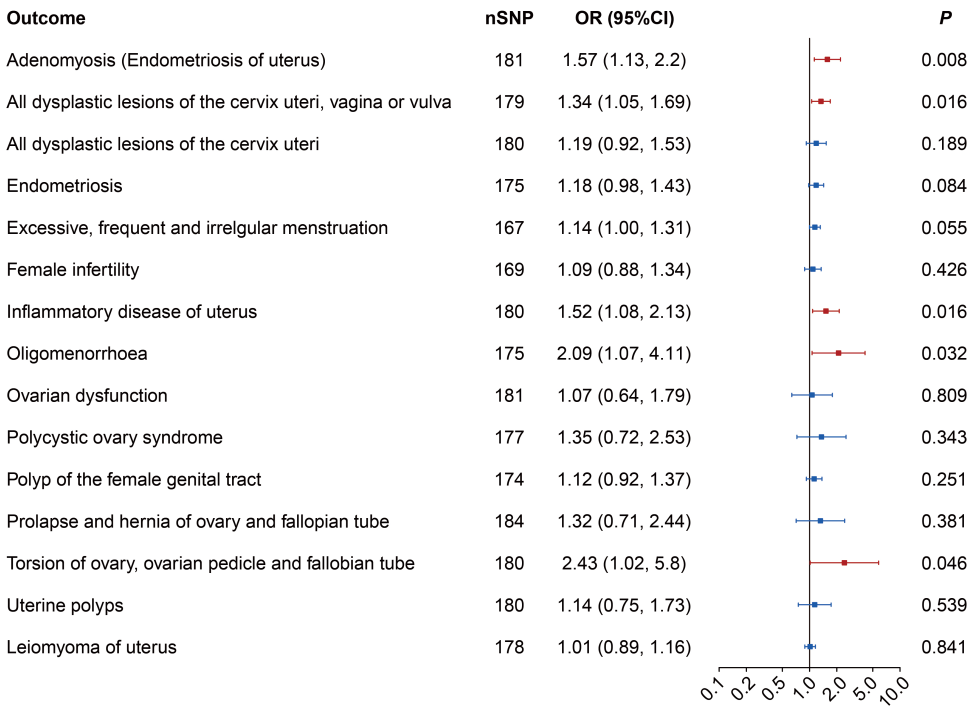

Figure 3. Forest plot of causal association between smoking initiation and female reproductive disorders. nSNP, number of single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

cessation and endometriosis (OR = 0.58, P = 0.015) among the 15 female reproductive disorders analyzed in the study (Figure 5). This finding was further supported by the weighted median (OR = 0.48, P = 0.016) and MR-RAPS (OR = 0.58, P = 0.017) approaches. Furthermore, the OR value, which was consistently less than one across all MR analysis methodologies, provided compelling evidence for the steadfastness and authenticity of the result (Supplementary Table 8).

Multiple approaches demonstrate the reliability of the findings

In all univariate MR analyses, none of the findings displayed any heterogeneity based on P-value of Cochran's Q statistic ([Q_P]; Q_P > 0.05 for all), and the MR Egger intercept (P > 0.05 for all) revealed no pleio-

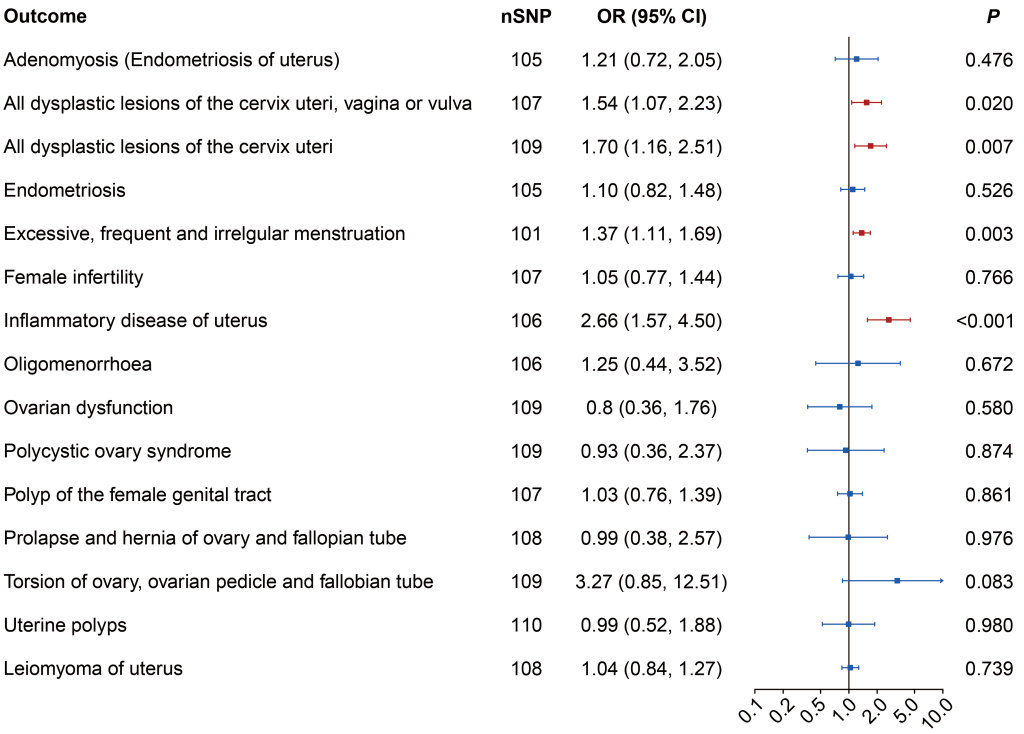

Figure 4. Forest plot of causal association between lifetime smoking index and female reproductive disorders. nSNP, number of single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

tropy. Furthermore, the leave-one-out analysis did not identify any SNPs with a substantially large impact on outcomes, and MR-PRESSO analysis did not detect any outliers. Additionally, the MR Steiger test used to investigate the causal hypotheses between three smoking phenotypes and female reproductive disorders confirmed the accurate causal direction of the association ($P < 0.001$ for all). These methods confirmed the stability and reliability of the results (Supplementary Table 6-8).

Multivariable Mendelian randomization

Given that alcohol consumption is a plausible risk factor for female reproductive disorders, we incorporated it into the MVMR analysis and examined the significant causal effects of smoking initiation and lifetime smoking index on female reproductive disorders identified in the univariate MR analysis, to determine whether these effects remain independent of alcohol consumption (Table 1). The findings indicated that the lifetime smoking index continued to be a crucial contributor to female reproductive disorders (OR = 1.49-2.44; $P < 0.05$ for all), even after accounting for alcohol intake. On the other hand, smoking initiation exhibited a causal association with only adenomyosis (OR = 1.71, $P = 0.014$) after accounting for alcohol intake. All MVMR analyses remained unaffected by heterogeneity (Q_$P > 0.05$ for all) and pleiotropy ($P_{\text{MR Egger intercept}} > 0.05$ for all), thereby confirming the robustness of the results (Supplementary Table 9).

Colocalization analysis

Colocalization analysis was conducted on all significant results from the univariate MR analysis. We found that for lifetime smoking index and inflammatory disease of the uteri, the PP.H4 for rs28485305 (chr15:74044197:C:T) and rs7528604 (chr1:66407352:G:A) were both greater than 75% (91.30% and 79.99%, respectively; Figure 6; Supplementary Table 10-12). The two SNPs correspond to the genes inhibitory synaptic factor 1 (*INSYN1*) and phosphodiesterase 4B (*PDE4B*), respectively, and are positively associated with the lifetime smoking index.

DISCUSSION

To the best of our knowledge, this is the first comprehensive study to investigate the causal relationship of smoking with a range of female reproductive disorders. Our findings revealed pervasive effects of smoking on female reproductive health, with different disorders being influenced at different stages of smoking, thereby significantly impacting female reproductive health.

In our findings, both smoking initiation and lifetime smoking index were causally associated with dysplastic lesions in the cervix uteri, vagina, or vulva, as determined through univariable MR. In an experimental study, cigarette smoke was shown to inhibit the regener-

Table 1: Multivariable Mendelian randomization analysis

Exposure	Outcome	OR	95% CI	*P*	Q_*P*	P^a	P^b	P^c	P^d
Lifetime smoking index	All dysplastic lesions of the cervix uteri	1.94	(1.29, 2.94)	0.002	0.996	0.005	0.002	0.136	0.754
Drinking per week		1.06	(0.67, 1.70)	0.799	0.996	0.413	0.799	0.845	
Lifetime smoking index	All dysplastic lesions of the cervix uteri, vagina, or vulva	1.79	(1.21, 2.63)	0.003	0.988	0.027	0.003	0.136	0.679
Drinking per week		1.12	(0.73, 1.73)	0.610	0.988	0.421	0.610	0.666	
Lifetime smoking index	Inflammatory disease of the uteri	2.44	(1.39, 4.26)	0.002	0.992	0.012	0.002	0.121	0.697
Drinking per week		1.33	(0.71, 2.49)	0.381	0.992	0.321	0.381	0.432	
Lifetime smoking index	Excessive, frequent, and irregular menstruation	1.49	(1.19, 1.86)	0.001	0.993	< 0.001	0.001	0.791	0.234
Drinking per week		0.88	(0.68, 1.14)	0.327	0.993	0.856	0.327	0.399	
Smoking initiation	Adenomyosis	1.71	(1.11, 2.62)	0.014	0.803	0.129	0.014	0.373	0.078
Drinking per week		0.74	(0.28, 1.96)	0.544	0.803	0.648	0.544	0.365	
Smoking initiation	All dysplastic lesions of the cervix uteri, vagina, or vulva	1.34	(0.98, 1.81)	0.063	0.999	0.123	0.063	0.573	0.975
Drinking per week		1.05	(0.53, 2.08)	0.897	0.999	0.929	0.897	0.902	
Smoking initiation	Torsion of ovary, ovarian pedicle, and fallopian tube	2.40	(0.77, 7.47)	0.132	0.995	0.142	0.132	0.485	0.821
Drinking per week		0.74	(0.06, 9.75)	0.819	0.995	0.113	0.819	0.855	
Smoking initiation	Oligomenorrhea	1.76	(0.74, 4.14)	0.199	0.992	0.547	0.199	0.053	0.106
Drinking per week		1.61	(0.22, 11.88)	0.64	0.992	0.841	0.640	0.488	
Smoking initiation	Inflammatory disease of the uteri	0.91	(0.34, 2.48)	0.857	0.982	0.614	0.857	0.275	0.053
Drinking per week		1.60	(1.04, 2.45)	0.033	0.982	0.231	0.033	0.007	

OR, odds ratio; CI, confidence interval; *P*, *P*-value of Multivariable inverse-variance weighted; Q_*P*, *P*-value of Cochran's Q statistic of Multivariable inverse-variance weighted; P^a, *P*-value of Multivariable Median; P^b, *P*-value of Multivariable LASSO; P^c, *P*-value of Multivariable Egger; P^d, *P*-value of Multivariable Egger Intercept.

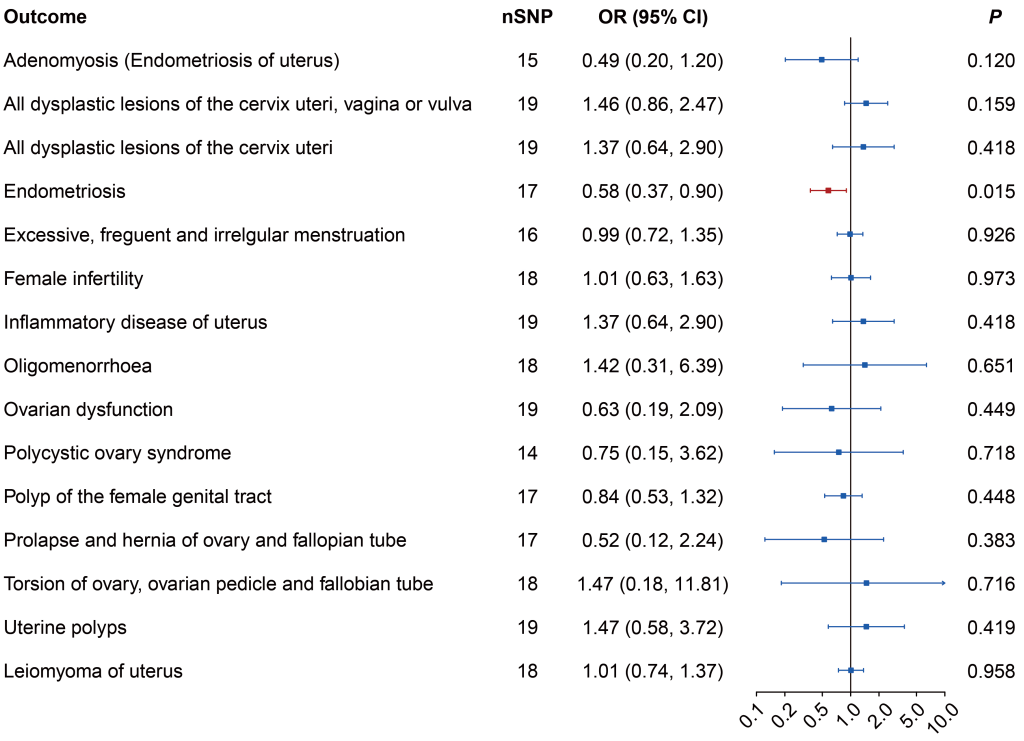

Figure 5. Forest plot of causal association between smoking cessation and female reproductive disorders. nSNP, number of single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

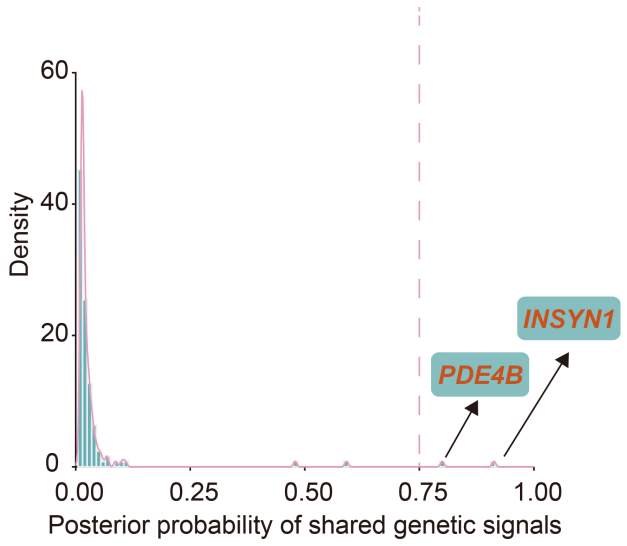

Figure 6. Distribution of the posterior probability for shared genetic loci between lifetime smoking index and inflammatory disease of the uteri. INSYN1, inhibitory synaptic factor 1; PDE4B, phosphodiesterase 4B.

ation of various tissues from stem cells in the reproductive system, resulting in tissue damage and functional impairment of the uteri and vagina.[32] Moreover, multiple reports have indicated the association of smoking with vulvar neoplasms,[33,34] vaginal neoplasms, and uterine cervical neoplasms.[35] These results imply that the impact of smoking may extend to the early stages of cancer development, advancing our comprehension of the mechanism by which smoking affects cancer growth. Early diagnosis and treatment of smokers may potentially arrest disease progression.

Furthermore, our study also uncovered the causal relationship between smoking and inflammatory diseases of the uteri. This finding is consistent with numerous previous studies. For instance, an observational study suggested that cigarette smoke inhalation causes a significant increase in inflammatory gene expression in female reproductive organs, resulting in an abnormal inflammatory response that may lead to the development of inflammatory diseases of the uteri.[9] Clinical studies have also indicated that prolonged smoking leads to the accumulation of chemical compounds that disrupt estrogen receptor levels in the uterus, resulting in inflammatory diseases.[36]

Colocalization analyses for the variants selected as the MR IVs highlighted the shared genetic signals between lifetime smoking index and inflammatory disease of the uteri, including loci near genes *INSYN1* and *PDE4B*. *PDE4B* belongs to a family of proteins called phosphodiesterases, which play a crucial role in metabolizing cyclic adenosine monophosphate within inflammatory cells.[37] This enzyme is recognized as a key modulator in the process of resolving inflammation.[38] Studies have established that *PDE4B* can alleviate smoking-induced inflammation, although the research has primarily focused on the lungs.[39–41] Simultaneously, studies have suggested the role of *PDE4B* in promoting uterine inflammation. For instance, in pregnant mice at the 15-day gestation stage, intrauterine injection of Escherichia coli Lipopolysaccharide led to an increase in phosphodiesterase 4 (PDE4) activity and *PDE4B* expression at the maternal-fetal interface. Inhibiting PDE4 prevented inflammation-induced preterm birth and fetal death.[42] Inhibitors targeting *PDE4B* have also been employed to achieve anti-inflammatory effects on the uterine myometrium.[42] Therefore, it can be speculated that smoking may induce uterine inflammation by activating intracellular *PDE4B*. However, further investigation is necessary to elucidate the precise mechanisms involved in this process.

It is evident that the duration of smoking, as indicated by the lifetime smoking index, has a more significant impact on dysplastic lesions in the cervix uteri, vagina, or vulva, as well as inflammatory disease of the uteri, compared to whether an individual had ever been a regular smoker. Furthermore, the impact of the lifetime smoking index on these two female reproductive disorders remained significant even after adjusting for alcohol intake in the MVMR analysis. This may be attributable to the presence of thousands of chemicals in tobacco smoke which once introduced into the body are not easily metabolized.[43] As a result, women who smoke for an extended period are exposed to high levels of chemicals in the body, which can potentially disrupt estrogen levels and cause tissue damage.[44,45]

However, our findings suggested no causal relationship of smoking with female infertility, which contradicts previous studies. For instance, a meta-analysis suggested that smoking can disrupt hormone balance, leading to sexual dysfunction and reproductive challenges in women.[46] A prospective observational study reported a 20% higher infertility rate among women who smoked for $\geqslant$ 10 years compared to those who never smoked.[47] Nevertheless, these findings should be interpreted with caution. Infertility, as defined, refers to the inability to achieve a clinical pregnancy after 12 months of regular unprotected sexual intercourse,[48] and it can be influenced by both male and female factors. However, it remains challenging to eliminate the influence of male factors in observational studies as well as MR analysis, and this may potentially result in divergent results. Furthermore, previous studies have indicated a link between smoking and PCOS.[10,49] However, in the

present study, we observed no conclusive causal relationship between any of the three smoking phenotypes and PCOS. Consequently, large-scale randomized controlled trials are required to assess the association between smoking and PCOS.

Our study has the following notable strengths. First, we utilized LDSC and MR methodologies to examine the genetic correlation and causal relationship between smoking and female reproductive disorders. Additionally, we validated the MR outcomes through colocalization analysis. Moreover, we employed multiple MR approaches to assess causal relationships and employed MVMR to eliminate the main confounding factor of alcohol intake, which improved the robustness of our findings. Second, our results were not impacted by heterogeneity and pleiotropy. Third, we utilized multiple smoking phenotypes to capture smoking status at various stages, thereby providing a more comprehensive understanding of the duration and intensity of smoking. The consistent associations between these phenotypes and multiple female reproductive disorders indicate the robustness of our results. Furthermore, we screened for a broader range of diseases with a larger number of cases, which allowed for a more comprehensive examination of the relationship between smoking and female reproductive disorders. However, despite these strengths, some limitations of our study should be considered while interpreting the results. The study population comprised exclusively of European participants, which may limit the generalizability of our findings to other populations. Furthermore, for each specific type of female reproductive disorder, we only included a single GWAS dataset, which may potentially limit the statistical power.

Several methodological limitations should also be acknowledged. First, although multiple sensitivity analyses were performed, residual horizontal pleiotropy cannot be completely excluded. Second, phenotype misclassification may exist in GWAS datasets because diagnostic criteria and phenotype definitions may vary across contributing cohorts. Third, Mendelian randomization relies on three core assumptions: the genetic instruments must be strongly associated with the exposure, independent of confounders, and influence the outcome only through the exposure. Any violation of these assumptions may affect causal interpretation. Therefore, the findings should be interpreted within the methodological scope of genetic epidemiological analyses and require further validation in independent datasets and mechanistic studies.

To summarize, this study expands upon previous research to demonstrate that smoking contributes to a range of female reproductive disorders, with the harmful effects showing a dose-dependent relationship. These findings underline the dangers of smoking in females and may contribute to reducing the number of smokers. Furthermore, *PDE4B* was identified as a potentially important mediator of smoking-induced uterine inflammation. Further in-depth research is necessary to unravel the underlying mechanisms, thus opening up new avenues for innovative treatment approaches.

DECLARATIONS

Supplementary Information
Supplementary materials are only available at the official site of the journal (www.hksmp.com).

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Author contributions
Liu ML and Tang SL: Methodology, Formal analysis, Investigation, Data curation, Writing—Original draft preparation, and Visualization. Wu HY, Wang LQ, Zhang YJ, Zhang HX, Wang Q, Wu H, Xu L, Yan YH, and Lu MS: Investigation, Validation, Data curation, and Resources. Tang XH: Conceptualization, Supervision, Project administration, Funding acquisition, and Writing—Review and Editing. Liu ML and Tang SL contributed software. All authors read and approved the final manuscript.

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Ethical approval
Not applicable.

Informed consent
Not applicable.

Conflict of interest
The authors declare no competing interest.

Use of large language models, AI and machine learning tools
No artificial intelligence (AI) tools or large language models (LLMs) were used in the design, conduct, analysis, or writing of this study.

Data availability statement
The GWAS summary statistics used in this study were obtained from publicly available datasets. No new

datasets were generated in this study. The code used for the analyses is available from the corresponding author upon reasonable request.

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